Int. J. Applied Bioresearch (2015) 23:1-6.
Article ID : ijab150315101
ISSN 2250 - 2033 (Print) e ISSN: 2250 - 2041 (Online)

OPEN ACCESS

Antimicrobial Activity of Plant Extracts against Some Human Diseases Causing Microorganisms

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Received: 31 January, 2015 / Accepted: 7 February, 2015 / Published Online: 15 March, 2015

Abstract

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. The aim of this study is to determine the antimicrobial activities of selected traditionally used medicinal plants such as *Ruta chalepensis, Lantana camara and Azadirachta indica* against standard reference bacterial and fungal species. The antimicrobial activity of leaves and seed of these plants were evaluated using Soxhelt extraction method. Methanol, Petroleum ether, Chloroform, Acetone and Ethanol were used as solvents in order to prepare the plant extracts. The antimicrobial activity was screened by using disc diffusion technique against pathogenic bacteria species of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* and *Salmonella typhi* and fungal species *Fusarium oxysporum*. The diameter of inhibition zone was measured in millimeters from the centre of the disc. The leaf extract were effective when compare to the seed extract. *S. aureus* was found to be the most resistant for all the plant extracts and solvents. Methanol and Ethanol extracts of *R. chalepensis* and Aceton extract of *L. camara* showed antibacterial and antifungal activity against disease causing organisms and this suggest that constituents of the plants could be useful in chemotherapy.

Key words: Antimicrobial, Antibacterial, Antifungal, Plant extracts, Zone of inhibition.

Citation: Leul Abebe, Samuel Sahile and Subramanian,C. 2015. Antimicrobial activity of plant extracts against some Human Diseases Causing Microorganisms. *Int. J. Applied Bioresearch*, 23:1-6.

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Manuscript Type : **Research Article**
Received Manuscript : **Via Email**
Approved Letter : Received or **Non Received**
Funding Source: Support or **Non Support**
Conflict of Interest : **Nil**
Manuscript Full Responses: **Authors**

Submission manuscripts info:
gbtrplink@gmail.com

1. Introduction

Nature has been a source of medical agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Douglas, 1987). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, of the photochemical constituents that have antimicrobial properties (Lewis and Ausubel, 2006). Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30 percent of the entire plant species, at one time or another is used for medicinal purposes (Sukhdev *et al*., 2008).

Among 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. Ethiopia is the origin or center of diversity for many of these plant species (Endashaw, 2007). The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants of which about 12 percent are endemic, however, many others are not yet identified. About 300 of these species are frequently mentioned in many sources (Fekadu, 2001).

Many efforts have made to discover new antimicrobial compound from various kind of sources such as soil, microorganisms, animals and plants. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases (Alam, 2009). Medicinal plants are finding their way into pharmaceuticals, cosmetics, neutraceuticals. Plants have given Western Pharmacopoeia about 7000 different pharmacologically important compounds and a number of top selling drugs of modern times eg. Quinine, taxol, campothecine etc. (Tshibangu *et al*., 2002).

Medicinal plants occupied an important position in the socio-cultural, spiritual and medicinal arena in Ethiopia. The various literature available show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70 percent of human and 90 percent of livestock population depend on traditional medicine similar to many developing countries particularly that of Sub-Saharan African countries (Abebe, 1996; Endashaw, 2007)..

Therefore, the aim of this study is to determine the antimicrobial activities of some selected traditionally used medicinal plants such as *Ruta chalepensis, Lantana camara* and *Azadirachta indica* against standard reference bacterial and fungal species

2. Materials and Methods

2.1 Samples collection

Fresh and healthy leaves and seeds of *Ruta chalepensis* and *Lantana camara* were collected from the field at University of Gondar. As the *Azadirachta indica* trees grows only around lower elevation in Ethiopia, the leaves and seeds of this plant was collected from Metema woreda located 180 kilometres north west of University of Gondar. The voucher specimens were identified by comparing herbarium at Botany laboratory of University of Gondar.

2.2 Preparation of Crude Extracts of plant materials

The seeds and leaves of each plants were washed thoroughly with running tap water and finally with sterile distilled water. Then, they were dried in an open air, protected from direct exposure to sunlight to prevent degradation of active ingredients (Pinkee *et al*., 2001). Then, the plant material were grounded using grinding machine (Kika-Werke GMBH, Germany) and passed through 250µm mesh sieve to obtain a fine powder. From the sieved powder sample 200g were extracted with 500 ml of absolute Petroleum ether at room temperature on a shaker (Bibby Sterilin Ltd., United Kingdom) with mild shaking for 72 hours. The extracts were filtered using filter paper (Whatman No 1) and the solvent fraction were evaporated on the rotary evaporator (Buchi Rota-Vapor RE-52, Switzerland) under reduced pressure at 77^0 C. The obtained extracts were further dried at room temperature and preserved.

The residue of Petroleum ether extraction, was further extracted with 500ml of chloroform and residues of chloroform extraction was extracted with 500ml of absolute acetone, residue of acetone extraction was extracted with 500ml of absolute methanol, and residue of methanol extraction was extracted with 500ml of absolute ethanol at room temperature for 72 hours on shaker. The extracts were filtered using filter paper (Whatman No 1) and the solvent were evaporated on the rotary evaporator under reduced pressure at 61^0 C; 56^0 C; 65^0 C and at 78^0 C respectively. The extracts were further dried at room temperature (Nostro *et al*., 2006).

2.3 Preparation of culture media

Mueller Hinton agar (MHA) (Sisco Research lab. pvt. Ltd.) and Sabauraud dextrose agar (SDA) media were used for direct sensitivity testing. The media was prepared and treated according to manufacturer's guidelines. 35 g for Mueller Hinton agar and 68g for SDA was mixed with one liter of distilled water separately, enclosed in a screw cap container and autoclaved at 121° C for 15 minutes. Each medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37° C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile.

2.4 Test Strains

Staphylococcus aureus (ATCC 2923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 9289) and *Klebsiella pneumonia* (ATCC 700603) all American Type Culture Collections were obtained from Gondar university Hospital. *Fusarium oxysporum* was obtained from Haramaya University plant protection laboratory.

2.5 Antimicrobial activity assay

Antimicrobial activity assay was determined using Kirby–Bauer method by disc diffusion method **(**Drago *et al*., 1999). Two mg of each extract was dissolved in three 0.2 ml of their corresponding solvents. The inocula of the respective bacteria and fungi were then spread on to the MHA and SDA plates respectively using a sterile swab to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. Duplicates were maintained for all inocula. The paper discs soaked within each of the extract, standard drug solution and the control-blank were placed separately in each quarter of the plate using sterile forceps. Multiple plates were (triplicate replications) done for each of the extract. The plates were then maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 37° C for 24 h for the bacteria and 25° C for 48 h for the fungus and the zones of inhibition were subsequently measured in mm (Mukherjee *et al*., 1995a, b). Tetracycline and Ketoconazole were used as positive control for the antibacteria and antifungal susceptibility test respectively.
The inhibition zone values were interpreted as sensitive (19 mm), intermediate (15-18 mm) and resistant (<14 mm) **(**Drago *et al*., 1999).

2.6 Determination of the Minimum Inhibitory Concentration (MIC) of the extract

Of the three plants test of their seed and leaf extract only those that showed antibacterial and antifungal activity were selected for further tests to calculate their MIC by dilution method. The MIC of the plant extracts were determined by diluting the raw extracts in saline solution to various concentrations. Equal volume of diluted extract and nutrient broth and Sabauraud dextrose broth for the bacteria and fungus respectively were mixed in a test tube and 0.2 ml of the suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated using standard loop. The tubes were incubated at 37° C for 24 hours for the bacteria and 25° C for 48 hours for the fungus. Two control tubes were maintained for each test batch that included as antimicrobial control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium and the inoculum) **(**Cheruiyot *et al*., 2009).

3. Results and Discussion

Result of the antimicrobial activates was summarized in following tables. The zones of inhibition produced by the test organisms indicated their susceptibility to the plant extracts; it was observed that the zones of inhibition varied from one organism to another and from one plant part extract to another. According to different literature the effect of an agent varies with target species. From all the crude extract methanol extract were effective followed by acetone, chloroform, ethanol and petroleum ether extract. The largest zone of inhibition was recorded by acetone extraction of *L. camara* leaf extract (25 mm) against *S. aureus* and the lowest was ethanl extraction of *R. chalepensi* leaf extract (7 mm) against *S. tayphi*.

The study results shows all the test organisms were either resistant or intermediate to all the extracts as Wafaa *et al*., (2007) reported the antibacterial and antifungal activities of the native and chemically modified extracts from *A. indica* seeds, seed-hulls and leaves. *A. indica* from different solvent of the plant except *K. pneumonia* was sensitive that was 20±0.3 mm to methanol extraction of the *A. indica* leaf (Table 1). Among different solvents used for the study revealed, that acetone extraction of *L. camara* exhibited antimicrobial activity against *E. coli* 23±0.9 mm and *S. aureus* 25±2.9 mm, Chloroform extraction against

Table -1: Antibacterial and antifungal activities of *A. indica*

Test organism	Plant Part	P. ether	Chloroform	Acetone	Methanol	Ethanol	Ketoconazole 0.3mg/ml
S.aureus	Leaf	10 ± 0.3	-	11±0.6	10±1.2	-	ND
	Seed	13 ± 1.8	-	9±1.4	11±1	12±1.6	ND
K.pneumonia	Leaf	7 ± 0.3	-	10±0.9	20±0.3	-	ND
	Seed	12 ± 1.2	11±0.7	11±1	-	9±1.2	ND
S.typhi	Leaf	13 ± 0.9	-	12±0.3	-	-	ND
	Seed	14 ± 0.9	10±1.2	-	12±0.6	-	ND
E.coli	Leaf	9 ± 0.3	-	11±1.4	10±1.2	-	ND
	Seed	10 ± 1.3	9±1.6	-	12±0.9	11±0.9	ND
P.aeruginosa	Leaf	8 ± 0.9	-	10±0.6	18	-	ND
	Seed	15 ± 0.6	10±0.9	10±1.7	15±0.5	13±0.6	ND
F.oxysporum	Leaf	12 ± 0.9	19±0.6	8±0.6	12±1	-	23±2.4
	Seed	10 ± 1.2	16±1.8	-	10±1.2	13±0.3	23±2.4

Note: - = no zone of inhibition, ND = Not Determined

Table 2. Antibacterial and antifungal activities of *L. Camara*

Test organism	Plant Part	P.ether	Chloroform	Acetone	Methanol	Ethanol	Ketoconazole 0.3mg/ml
S.aureus	Leaf	-	13±0.6	25±2.9	16±0.3	16±0.3	35±0.3
	Seed	-	12±0.9	11±0.6	12±0.3	10±1.2	
K.pneumonia	Leaf	-	12±0.3	12±1.2	-	10±0.6	25±1.2
	Seed	-	8±0.3	13±0.6	9±0.3	-	
S.typhi	Leaf	-	13±2	-	12±0.6	14±0.3	35±0.6
	Seed	-	14±0.6	10±0.9	12±0.7	9±0.7	
E.coli	Leaf	-	14±0.3	23±0.9	13±0.9	14±1.4	30±0.3
	Seed	-	-	11±0.3	11±0.9	12±0.9	
P.aeruginosa	Leaf	-	10±1.2	16±0.6	9±1.2	11±0.3	26
	Seed	-	8±0.6	8±0.6	8±0.3	-	
F.oxysporum	Leaf	9±1	25±1.4	-	9±1	14±0.3	ND
	Seed	9±4.8	13±0.9	9±0.6	8±0.3	-	

Note: - = no zone of inhibition, ND = Not Determined

Table 3. Antibacterial and antifungal activities of *R. chalepensi*

Test organism	Plant Part	P. ether	Chloroform	Acetone	Methanol	Ethanol	Cont Ketoconazole 0.3mg/ml
S.aureus	Leaf	-	12±0.6	20±0.8	11±0.6	13±0.9	35±0.3
	Seed	9±0.9	11±0.7	9±0.9	20±1.6	24±0.3	
K.pneumonia	Leaf	13±2.9	16±1.2	13±1.3	15±0.6	14±0.6	25±1.2
	Seed	-	20±0.6	11±1	16	18±0.6	
S.typhi	Leaf	-	16±0.6	14±1.8	19±1.2	7	35±0.6
	Seed	-	15±1.7	8	19±1.6	20±0.9	
E.coli	Leaf	9±1	12±0.6	13±0.9	11±0.6	19±0.3	30±0.3
	Seed	-	9±1.7	13±0.6	8±0.9	16±1.2	
P.aeruginosa	Leaf	-	8±0.6	19±0.3	24±0.9	14±0.7	26
	Seed	-	15±0.6	12±0.6	24±2	15±0.7	
F.oxysporum	Leaf	12±1.2	13±0.7	11±1.4	-	11±0.3	ND
	Seed	13±1.8	12±1.8	9±0.6	9±0.9	16±2	

F. oxysporum 25±1.4 mm (Table 2). There are several evidences on the presence of antimicrobial metabolites like tannins, flvonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids, etc in the studied plant species (Ghisalberti, 2000; Ross, 1999). It was presumed that the presence of flavonoid may be contributing the analgesic activity of acetone extraction (Table 2).

Methanol extract of *R. chalepensi* leaf shows high zone of inhibition on *P. aeruginosa* 24±0.9 mm, *S. typhi* 19±1.2 mm, ethanol extraction on *E. coli* 19±0.3 mm and the acetone extraction on *P. aeruginosa* 19±0.3 mm, *S. aureus* 20±0.8 mm. Ethanol and methanol extract of *R. chalepensi* seed show high zone of inhibition against *S. aureus* 24±0.3 mm, 20±1.6 mm, *S. typhi* 20±0.9 mm, 19±1.6 mm respectively and Chloroform extraction on *K. pneumonia* 20±0.6 mm P. ether extract of *R. chalepensi* seed showed no antibacterial activity against *P. aeruginosa, S. typhi, E. coli* and *K. pneumonia* (Table 3).

Leaf extract were effective when compare to the seed extract. *S. aureus* was found to be the most resistant followed by *K. pneumonia*, *P. aeruginosa, E. coli, S. typhi* and *F. oxysporum*. In fact, gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market. The MIC for *S. typhi* and *P. aeruginosa* were almost half of that of *S. aureus* showed more susceptibility to the activity of the crude extract of *S. aureus*. The activities of the crude extract are more against Gram positive bacteria compare to Gram negative bacteria.

Bassam *et al*., (2008), Sathiyapriya *et al*., (2009), Lakshmana *et al*., (2011) had worked on *Azadirachta indica, Ruta chalepensis* and *Lantana camara* respectively and their result were in contrary to the present results. This may be due to the known botanical fact that trees from different locations may demonstrate different properties since they are grown on different soils and in varying climates and are exposed to different chemical and biologic flora. Not only in biochemical property and also the chemical compositions of the concerned plants parts or tissues can be influenced by different origins, environmental, and seasonal factors as well as their superior equipment and extraction methods where in the active component of the leaf was better isolated, Moreover, the effectiveness of the extracts varies with its concentration and the kind of bacteria used in the study.

4. Acknowledgements

We greatly acknowledge the Gondar university Hospital and Haramaya University plant protection laboratory for providing the test strains.

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